

ON THE LACK OF ANTAGONISM BETWEEN CALCIUM VERSUS MAGNESIUM AND ALSO BETWEEN CALCIUM VERSUS SODIUM

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(WITH TWO FIGURES)

In a former paper,¹ I have shown that many of the facts relating to the toxic and antitoxic effects of salt for animals and plants, as shown by the work of LOEB and OSTERHOUT in particular, hold in a general way with regard to the ammonifying power of *Bacillus subtilis*. In the same paper I also reported the results of experiments which showed that there is in respect to the ammonification by *B. subtilis* no antagonism between calcium and magnesium. As this is so striking an exception to the results obtained by LOEW² in his well-known experiments on green plants, it was thought advisable to emphasize the writer's results in a separate article, along with another new and strikingly exceptional case, namely a lack of antagonism between calcium and sodium. The latter case is of great interest, inasmuch as KEARNEY and CAMERON,³ BENECKE,⁴ and OSTERHOUT⁵ have all found very pronounced antagonism between sodium and calcium in green plants. OSTERHOUT has also found this to be the case for a mold (*Botrytis*).⁶

In general, the technic employed in the two sets of experiments reported was as follows: Solutions of chemically pure salts, previously submitted to the flame test, were made up at a concentration of 0.35*m* containing 0.75 per cent. Witte's peptone. Inoculations were made from a 48-hour peptone culture into solutions made up as described in detail below. At the end of the incubation period of 2.5 days at

¹ BOT. GAZETTE 48:105-125. 1909.

² Literature in LOEW, Bull. 18, Div. Veg. Physiol. and Path., U. S. Depart. Agric. 1899; also LOEW and ASO, Bull. Coll. Agric. Tokyo 7:395. 1907.

³ Report 71, U. S. Depart. Agric. 1902.

⁴ Ber. Deutsch. Bot. Gesells. 25:322. 1907.

⁵ BOT. GAZETTE 42:127. 1906; 44:259. 1907; Jahrb. Wiss. Bot. 46:121. 1908.

⁶ Univ. Calif. Publ. Botany 2:317. 1907.

28–29° C., the culture solutions were transferred to Jena distillation flasks and distilled after adding a slight excess of magnesium oxid. The distillate (about 150^{cc} in bulk) was titrated against tenth normal acid, cochineal being used as the indicator, and the amount of ammonia nitrogen formed thus determined. Sterile controls were run on all determinations to allow of an accurate estimation of the ammonia actually formed by *B. subtilis* during the period of incubation.

Series I. CaCl₂ versus MgCl₂

Solutions of calcium chlorid and magnesium chlorid of 0.35^m each were made up in bulk to contain 0.75 per cent. peptone. The culture solutions were made and placed in 250^{cc} Erlenmeyer flasks as follows: Flask *A* contained 100^{cc} of the MgCl₂ solution made up as noted above; flask *B*, 100^{cc} MgCl₂ + 5^{cc} CaCl₂; flask *C*, 100^{cc} MgCl₂ + 10^{cc} CaCl₂; flask *D*, 100^{cc} MgCl₂ + 25^{cc} CaCl₂; flask *E*, 100^{cc} MgCl₂ + 50^{cc} CaCl₂; flask *F*, 100^{cc} MgCl₂ + 100^{cc} CaCl₂. Then beginning at the other end of the series, flask *K* contained 100^{cc} of the CaCl₂ solution; flask *J*, 100^{cc} CaCl₂ + 5^{cc} MgCl₂; flask *I*, 100^{cc} CaCl₂ + 10^{cc} MgCl₂; flask *H*, 100^{cc} CaCl₂ + 25^{cc} MgCl₂; flask *G*, 100^{cc} CaCl₂ + 50^{cc} MgCl₂.

After the solutions were made up, the mixed solutions in each flask amounted to more than 100^{cc}, while the flasks with pure salts (plus peptone) contained only 100^{cc}. In order to make the conditions the same for all the flasks (particularly in respect to the amount of fluid surface exposed to the air), enough liquid was pipetted out of the flasks containing mixed solutions to leave just 100^{cc} in each flask. The flasks were then sterilized in the autoclave at 1.25 atmospheres of pressure for 30 minutes. A 48-hour culture of *B. subtilis* in 1 per cent. peptone was used as inoculating material. By slight shaking the membrane formed at the surface was precipitated to the bottom, and by tilting the flask to one side and carefully setting it down again, one part of the bottom of the flask remained free of membranous material and the liquid above was homogeneous in character. Of this homogeneous liquid 1^{cc} was taken for inoculation in each of the flasks prepared as above described. The table of results follows:

TABLE I

Numbers in first column refer to c.c. of 0.35*m* solutions

| Culture solution | Corresponding points on curve | N as NH ₃ formed in cultures, in mg |
|----------------------------------------------------------|-------------------------------|------------------------------------------------|
| 100 MgCl ₂ | A | 3.08 |
| 100 MgCl ₂ } 5 CaCl ₂ } | B | 2.59 |
| 100 MgCl ₂ } 10 CaCl ₂ } | C | 1.68 |
| 100 MgCl ₂ } 25 CaCl ₂ } | D | 0.98 |
| 100 MgCl ₂ } 50 CaCl ₂ } | E | 0.21 |
| 100 MgCl ₂ } 100 CaCl ₂ } | F | 0.07 |
| 50 MgCl ₂ } 100 CaCl ₂ } | G | 0.00 |
| 25 MgCl ₂ } 100 CaCl ₂ } | H | 0.00 |
| 10 MgCl ₂ } 100 CaCl ₂ } | I | 0.00 |
| 5 MgCl ₂ } 100 CaCl ₂ } | J | 0.00 |
| 100 CaCl ₂ | K | 0.49 |

By an examination of the curve drawn on the basis of table I (*fig. 1*), we are confronted by the very striking instance of no antagonism between the two salts employed. On the contrary, there is a constant increase of the toxic properties of each when the other is added to it in increasing amounts. In this exceptional behavior, so far as I can ascertain, *B. subtilis* (and probably all the ammonifiers) stand alone when their physiological efficiency in such salt mixtures is compared with that of the higher plants and animals. No instance of such behavior on the part of any member of the latter two groups of organisms has come to my notice in reviewing the results of similar researches on animals and the higher plants.

We find among plants the well-known researches of LOEW⁷ and his pupils, and later the researches of KEARNEY and CAMERON,⁸ which show the strong antagonism between calcium and magnesium.

⁷ Bull. 45, Bureau Pl. Ind., U. S. Depart. Agric.; also LOEW and Aso, Bull. Coll. Agric. Tokyo 7: no. 3. 1907.

⁸ Report 71, U. S. Depart. Agric.

The last-named investigators found in their experiments with the white lupine (*Lupinus albus*) and with alfalfa (*Medicago sativa*) that when CaCl_2 was added to MgSO_4 in about equal proportions the plants exhibited about 160 times the tolerance for the latter salt

that they did in solutions of MgSO_4 alone. They found further that the antagonism between CaCl_2 and MgCl_2 , though not so great (increasing the tolerance about 40 times), was nevertheless very marked, and

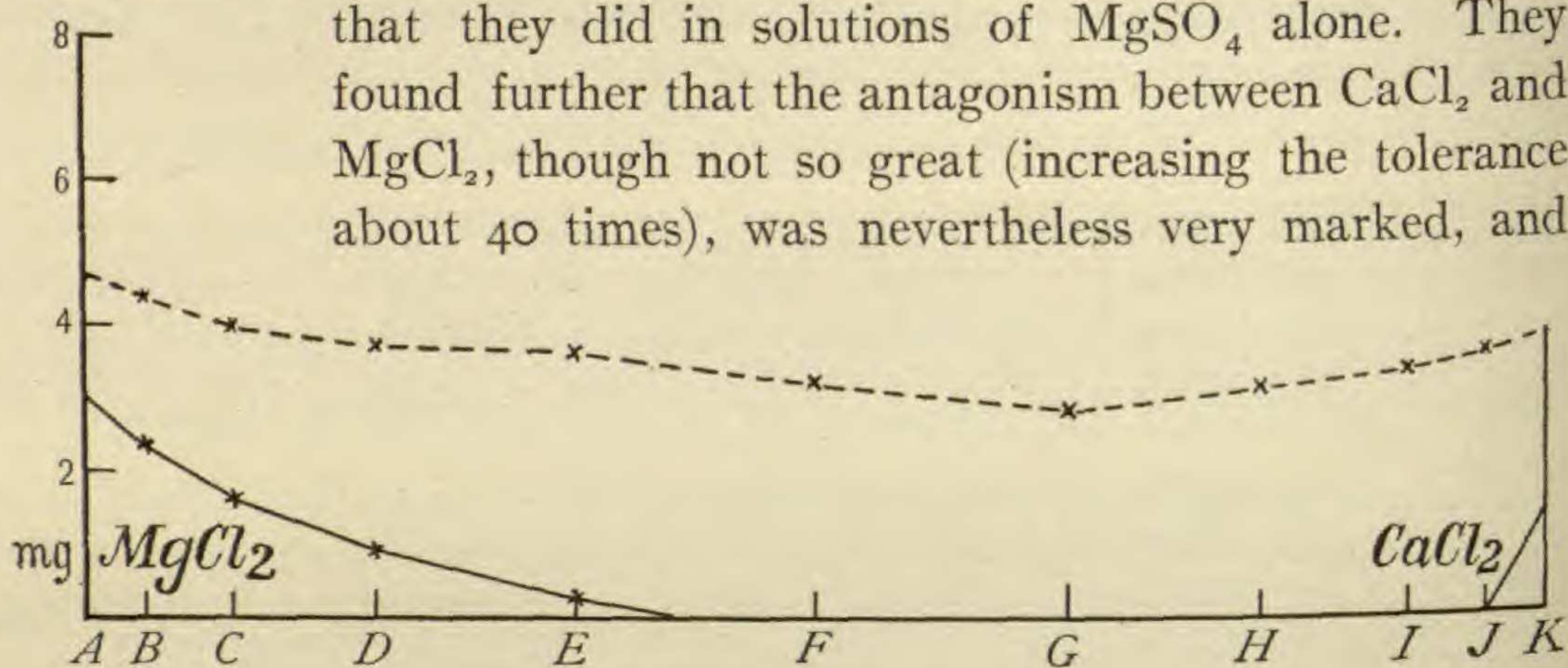


FIG. 1.—The ordinate at A represents the amount of ammonia nitrogen in milligrams formed in a pure 0.35*m* solution of MgCl_2 containing 0.75 per cent. peptone. The ordinate at K represents the amount of ammonia nitrogen formed in a pure CaCl_2 solution of the same strength and with the same peptone content. The ordinates at the intermediate points represent the amounts of ammonia nitrogen formed in various combinations of the two salts as indicated in tables I and II. The full line represents results in table I; the broken line represents results in table II.

where CaSO_4 replaced CaCl_2 the antagonism was very much greater between Ca and Mg than in either of the cases above cited. BENECKE⁹ likewise found antagonism between magnesium and calcium in his work with *Spirogyra*.

An antagonism between CaCl_2 and MgCl_2 , though slight, was found to be none the less definite by LOEB¹⁰ in experiments which showed that sea urchin blastulae and gastrulae would swim about in a mixture of the salts above mentioned for 48 hours, while each salt by itself would immediately prove poisonous at the concentration employed in the combination. Another interesting case in point may be noted in the experiments of the same investigator on *Polyorchis*,¹¹ a jellyfish of San Francisco Bay. In a solution of 50^{cc} NaCl + 6^{cc} MgCl_2 + 1^{cc} CaCl_2 , the rhythmical contractions of the margin go

⁹ Ber. Deutsch. Bot. Gesells. 25:322. 1907.

¹⁰ Amer. Jour. Physiol. 3:327. 1900.

¹¹ Jour. Biol. Chem. 1:427. 1906.

on normally, but with a slight increase of CaCl_2 the contractions are inhibited, and when 5^{cc} of a 0.375*m* solution of CaCl_2 are added, they are completely suppressed. On the other hand, when the margin of the fish, containing the sense organs and the central nervous system, is cut off, CaCl_2 exercises a stimulating action on the isolated center of *Polyorchis* and contractions go on normally; but when MgCl_2 is added to the solution in the ratio of 4 parts MgCl_2 to 1 part CaCl_2 , the stimulating action of the CaCl_2 is suppressed and contractions cease. In both cases, therefore, there is evidence of a definite antagonism between calcium and magnesium. LILLIE¹² also proved the existence of antagonism between the two salts when he found that the ciliary activity of the larvae of *Arenicola* would go on normally for some time in a mixture of approximately 4 parts MgCl_2 to 1 part CaCl_2 , whereas it would cease immediately if either of the salts at the same concentration was present alone. The same investigator found in other work that calcium salts inhibit the spontaneous contractility of the swimming plate in ctenophores, when added to solutions made up of 90 parts by volume NaCl and 10 parts MgCl_2 . Only 2 volumes of CaCl_2 of the same concentration as the other salts inhibit movements of the swimming plate in the sodium and magnesium solution above described, while 4 volumes of CaCl_2 allow but little spontaneous movement. The latter is entirely inhibited by the addition of 8 volumes of the CaCl_2 solution.

I wish to cite only one more case, which emphasizes by contrast most strongly the exceptional results obtained above in experiments with *B. subtilis*, and that is, the remarkable results obtained in a highly ingenious series of experiments recently carried out by MELTZER and AUER¹³ on the antagonistic effect of calcium on the inhibitory effect of magnesium. The experiments were carried out on rabbits, and in one case on a monkey, and as a typical instance of the remarkable antagonistic effect between calcium and magnesium may be cited the first experiment of the series, in which about 13^{cc} of an *m*/1 solution of MgCl_2 was injected subcutaneously into a rabbit. Less than one-half hour later there was produced general anaesthesia, with all the attending symptoms, and a 0.125*m* solution of

¹² Amer. Jour. Physiol. 5:56. 1901.

¹³ Amer. Jour. Physiol. 21:403. 1909.

CaCl₂ was injected intravenously in the ear vein. When only 2^{cc} had thus been injected, the rabbit was again breathing normally, and when 8^{cc} had been given, the animal sat up and appeared entirely recovered, except for a stiffness in the hind legs.

In these experiments, some of which were even more striking than the one cited, MELTZER and AUER employed, besides the chlorids of calcium and magnesium, the acetate and nitrate of the former, and the acetate, nitrate, and sulfate of the latter, and the same strong antagonism was noted in all cases.

In addition to the confirmation of the results obtained above in my experiments with the same material, one series was also carried out with a culture of *B. subtilis* obtained from an entirely different source, and the salt solutions made up from a different grade of chemically pure salt. As can be seen from the following table, the results fully

TABLE II

Numbers in first column refer to c.c. of 0.35*m* solutions

| Culture solutions | Corresponding points on curve | N as NH ₃ formed in cultures, in mg |
|----------------------------------------------------------|-------------------------------|------------------------------------------------|
| 100 MgCl ₂ | A | 4.76 |
| 100 MgCl ₂ } 5 CaCl ₂ } | B | 4.48 |
| 100 MgCl ₂ } 10 CaCl ₂ } | C | 4.20 |
| 100 MgCl ₂ } 25 CaCl ₂ } | D | 3.78 |
| 100 MgCl ₂ } 50 CaCl ₂ } | E | 3.64 |
| 100 MgCl ₂ } 100 CaCl ₂ } | F | 3.22 |
| 50 MgCl ₂ } 100 CaCl ₂ } | G | 3.08 |
| 25 MgCl ₂ } 100 CaCl ₂ } | H | 3.29 |
| 10 MgCl ₂ } 100 CaCl ₂ } | I | 3.43 |
| 5 MgCl ₂ } 100 CaCl ₂ } | J | 3.57 |
| 100 CaCl ₂ | K | 3.78 |

confirm those above given, and though the absolute amounts are different, the results are relatively the same (see also *fig. 1*).

It may be of interest to note here that *B. subtilis* from a 24-hour peptone agar slope culture was examined in hanging drops of molecular solutions of calcium chlorid and showed no perceptible ill-effects from the action of the solution. The ciliary movements appeared normal even after 24 hours in the hanging drop. It was noticed, however, that there was little or no division among the bacilli during the 24 hours, and it is likely that the calcium and magnesium salts exercise their toxic effects by inhibiting reproduction, since the ciliary movements seems to go on without interruption. These remarks, however, are based on too meager experimental evidence to partake of anything else than the nature of conjecture, but they serve to indicate a field of most interesting possibilities in research.

Though they are not analogous instances of the lack of antagonism between calcium and magnesium as shown above, it is interesting to note two cases on record, in which the addition of one salt to another made a combination more toxic than either. One case is that of OSTWALD'S¹⁴ experiments on the fresh-water Gammarus, in which it was found that a combination of $MgCl_2$ and $NaCl$ in solution was more toxic to that animal than $NaCl$ in solution alone. The other case is that noted in the experiments of PAUL and KRÖNIG,¹⁵ who found that the value of mercuric sulfate, acetate, and nitrate as disinfectants was enhanced by the addition of small amounts of the chlorids of potassium and sodium; but, on the other hand, the addition of the same chlorids to $HgCl_2$ reduced considerably the disinfecting power of the latter. The first instance is not analogous to my results, because one of the salts used by OSTWALD was different and the experiment was carried out under conditions so totally different that the value of a comparison here is doubtful. In the second instance, as PAUL and KRÖNIG themselves suggest, the increase of toxicity is not necessarily owing to a lack of antagonism between the two salts, but rather to the formation of complex double salts of mercury, characteristic of that element, and therefore this again cannot be compared with the lack of antagonism between calcium and magnesium above noted.

¹⁴ Univ. Calif. Publ. Physiology 2:163. 1905.

¹⁵ Zeitschr. Hyg. und Infectiouskrank. 25:57. 1897.

Series II. CaCl_2 versus NaCl

In this series the solutions were made up in the same way as in the preceding one, and the same concentration of salts and peptone was employed, the only difference being that CaCl_2 and NaCl were tested instead of CaCl_2 and MgCl_2 . The incubation was carried out for two and one-half days at $28-29^\circ \text{C}$., and the distillations and determinations were made as above described. The table of results follows:

TABLE III

Numbers in first column refer to c.c. of 0.35*m* solutions

| Culture solutions | Corresponding points on curve | N as NH_3 formed in cultures, in mg |
|----------------------------------------------------|-------------------------------|----------------------------------------------|
| 100 NaCl | A | 14.48 |
| 100 NaCl } 5 CaCl_2 } | B | 10.94 |
| 100 NaCl } 10 CaCl_2 } | C | 8.46 |
| 100 NaCl } 25 CaCl_2 } | D | 8.18 |
| 100 NaCl } 50 CaCl_2 } | E | 6.64 |
| 100 NaCl } 100 CaCl_2 } | F | 5.99 |
| 50 NaCl } 100 CaCl_2 } | G | 2.54 |
| 25 NaCl } 100 CaCl_2 } | H | 1.53 |
| 10 NaCl } 100 CaCl_2 } | I | 0.07 |
| 5 NaCl } 100 CaCl_2 } | J | 0.00 |
| 100 CaCl_2 | K | 0.00 |

In the curve drawn on the basis of the foregoing table (*fig. 2*) we note again the striking instance of the lack of antagonism for *B. subtilis* of two salts which showed a strong antagonism in all experiments on animals and plants thus far carried out. OSTERHOUT¹⁶ showed, for example, that a strong antagonism exists between NaCl and CaCl_2 for wheat, and that the curve obtained there was very similar

¹⁶ BOT. GAZETTE 48:98-104. 1909.

to the curve obtained for the antagonism between KCl and CaCl_2 . This latter fact is especially interesting, since for *B. subtilis* a strong antagonism is exhibited between KCl and CaCl_2 , while in the case of NaCl and CaCl_2 there is a constant increase of toxicity as CaCl_2 is added in larger and larger amounts to the NaCl solutions.

In this striking behavior *B. subtilis* is exceptional, not only as compared with a great variety of the higher plants, including liverworts, equisetum, algae, and some fungi, but also as compared with animals. LOEB¹⁷ found a marked antagonism between sodium and calcium ions in his studies on the development of animals. MOORE¹⁸ showed the same to hold true for contraction of the lymph hearts of the frog, and

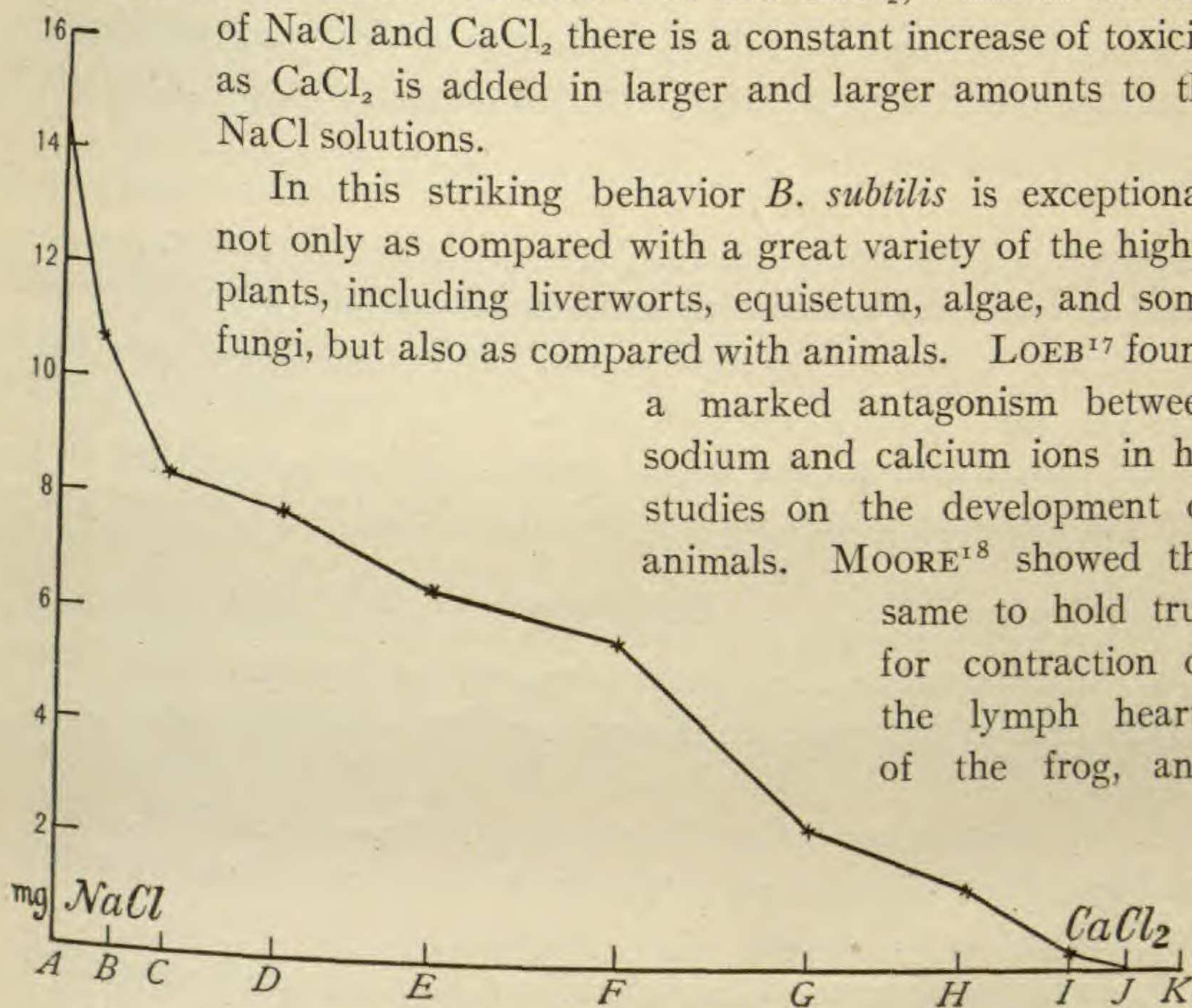


FIG. 2.—The ordinate at A represents the amount of ammonia nitrogen formed in a pure 0.35*m* solution of NaCl containing 0.75 per cent. peptone. The ordinate at K represents the amount of ammonia nitrogen formed in a pure 0.35*m* solution of CaCl_2 and with the same content of peptone. The ordinates at the intermediate points represent the amounts of ammonia nitrogen formed in various combinations of the two salts as indicated in table III.

LINGLE¹⁹ also demonstrated that fact in the case of the turtle's heart. In addition to these facts, we have the work of LILLIE²⁰ to show also that as regards ciliary activity an antagonism between CaCl_2 and NaCl was found to exist, and MCCALLUM in his work on cathartics showed the same facts to hold true there.

Many more instances could be cited to show by contrast the strikingly exceptional behavior of *B. subtilis* as regards the two salts

¹⁷ Amer. Jour. Physiol. 3:328, 383. 1909.

¹⁸ Ibid. 5:87. 1901.

¹⁹ Ibid. 4:265. 1900.

²⁰ Ibid. 5:56. 1901.

in question, but only one important case comes to my notice which resembles that of *B. subtilis* just cited, and that is to be found in the work of LOEB with the marine Gammarus, in which he found that the animals die more quickly in a solution of 100^{cc} 0.375^m NaCl to which 1^{cc} of a 0.375^m CaCl₂ solution was added than in a pure 0.375^m NaCl solution.

The results set forth in the foregoing pages, as well as those contained in a previous paper, tend to show that in their behavior toward salts bacteria differ in some respects from both plants and animals and occupy a position by themselves. It is evident that mineral fertilizers applied to the soil will not have altogether the same effect on the bacteria as on the higher plants. Further studies in this direction may lead to important practical application.

Summary

1. No antagonism exists between magnesium and calcium. Any combination of the two salts is more toxic than MgCl₂ alone for *B. subtilis*.

2. No antagonism exists between sodium and calcium. Any combination of the two salts renders it more poisonous than the NaCl alone for *B. subtilis*.

3. In these two respects, the behavior of *B. subtilis* finds no parallel among plants so far as studied, and scarcely any among animals.

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